

WE CLAIM:

1. A composition comprising a sustained culture of chicken embryonic stem cells with a transgene stably integrated into the genome of substantially all of the progeny of the embryonic stem cells and wherein the embryonic stem cell progeny contribute to a somatic tissue of a chimeric chicken produced by the injection of the ES cell progeny into a chicken embryo.

2. The composition of claim 1 further comprising a feeder layer of cells at a concentration of between approximately 10^3 and 10^5 cells/cm².

3. The composition of claim 2 wherein the concentration of feeder layer cells is less than 10^4 .

4. The composition of claim 2 or 3 wherein the layer of feeder cells are STO cells.

5. The composition of claim 1 further comprising BRL conditioned media.

6. The composition of claim 1 further comprising fetal bovine serum.

7. The composition of claim 1 wherein pluripotency is sustained for at least 180 days.

8. The composition of claim 1 wherein pluripotency is maintained at least 360 days.

9. A composition comprising a culture of chicken cells with a transgene is stably integrated in the genome of the cells, wherein the cells express an embryonic stem cell phenotype after being sustained in culture for more than 60 days.

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10. The composition of claim 9 wherein the integrated transgene encodes an exogenous protein.

11. The composition of claim 10 wherein the integrated transgene is greater than 15 kb.

12. The composition of claim 10 wherein the integrated transgene is greater than 60 kb.

13. The composition of claim 10 wherein the transgene encodes an exogenous protein present in a somatic tissue of chimeric chickens hatched following injection of the cells into a recipient embryo.

14. The composition of claim 13 wherein the transgene is expressed in a somatic tissue of chimeric chickens hatched from injection of the cells into a recipient embryo.

15. The composition of claim 14 wherein the somatic tissue is mesodermal.

16. The composition of claim 15 wherein the mesodermal tissue is lymphocytes.

17. The composition of claim 15 wherein the mesodermal tissue is oviduct.

5 18. The composition of claim 14 wherein the somatic tissue is mesodermal and endodermal.

19. The composition of claim 18 wherein the mesodermal and endodermal tissue is liver.

10 20. A composition of transgenic chicken embryonic stem cells in culture, wherein embryonic stem cell progeny contain a stably integrated transgene comprised of an operably-linked promoter, a selectable marker, and DNA encoding an exogenous protein, and wherein the embryonic stem cell progeny maintain pluripotency for more than 60 days such that injection
15 into a recipient embryo yields a chimeric chicken expressing the exogenous protein in B lymphocytes of the chimera.

21. A chimeric chicken comprised of embryonic stem cell progeny having a stably integrated transgene.

20 22. The chimeric chicken of claim 21 wherein the transgene is further comprised of an operably-linked promoter and a selectable marker.

23. The chimeric chicken of claim 22 wherein the transgene is further comprised of DNA encoding an exogenous protein.

24. The chimeric chicken of claim 21 wherein the transgene is comprised of a region
5 of homology to an endogenous chicken genome sequence.

25. The chimeric chicken of claim 23 wherein the size of the transgene is greater than
15 kb.

26. The chimeric chicken of claim 23 wherein the size of the transgene is greater than
10 100 kb.

27. The chimeric chicken of claim 23 wherein the exogenous protein is detected in
somatic tissue of the chicken.

15 28. The chimeric chicken of claim 27 wherein the exogenous protein is expressed in
somatic tissue of the chicken.

29. The chimeric chicken of claim 28 wherein the somatic tissue exogenous protein is
20 in mesodermal.

30. The chimeric chicken of claim 23 wherein the mesodermal tissue is lymphocytes
of the chicken.

31. A transgenic chicken having a genome comprised of a transgene of greater than 15 kb of exogenous DNA.

5 32. The transgenic chicken of claim 31 wherein the transgene is further comprised of an operably-linked promoter and a selectable marker.

33. The transgenic chicken of claim 31 wherein the transgene encodes an exogenous protein.

10 34. The transgenic chicken of claim 31 wherein the exogenous protein is expressed in somatic tissue.

15 35. The transgenic chicken of claim 31 wherein the somatic tissue is mesodermal.

36. The transgenic chicken of claim 31 wherein the mesodermal tissue is oviduct.

37. The transgenic chicken of claim 31 wherein the oviduct is lymphocytes.

20 38. The transgenic chicken of claim 34 wherein the somatic tissue is mesodermal and endodermal.

39. The transgenic chicken of claim 34 wherein the somatic tissue is liver.

40. The transgenic chicken of claim 31 wherein the chicken is an offspring of two chimeric chickens produced from sustained cultures of avian embryonic stem cells.

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41. A method of creating a chimeric chicken comprising:

selecting chicken embryonic stem cells from a culture sustained for more than 60 days wherein the embryonic stem cells have a genome comprising a stably integrated transgene, injecting the stem cells into a recipient embryo, and

hatching a chimeric chicken from the recipient embryo wherein the transgene is present in somatic tissue of the chimera.

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42. The method of claim 41 wherein the transgene encodes an exogenous protein.

43. The method of claim 42 wherein the exogenous protein encoded by the transgene is expressed in the somatic tissue of the chimera.

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44. The method of claim 43 wherein the somatic tissue is mesodermal tissue.

45. The method of claim 44 wherein the mesodermal tissue is lymphocytes of the chimera.

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46. The method of claim 41 wherein the transgene is comprised of a promoter and an operably-linked selectable marker.

47. The method of claim 41 wherein the culture is sustained more than 60 days.

48. The method of claim 41 further comprised of the step of breeding two of the chimeras.

49. A method to produce a chimeric chicken having expression of an exogenous protein in somatic tissue comprising:
preparing a sustainable culture of chicken embryonic stem cells,
transfecting the embryonic stem cells with a transgene encoding an exogenous protein,
selecting embryonic stem cells in which the transgene is stably integrated into the genome,
injecting the embryonic stem cells into a recipient embryo;
hatching the chimeric chicken wherein the somatic tissue of the chimeric chicken expresses the exogenous protein.

50. The method of claim 49 further comprising the step of breeding two of the chimeric chickens to produce a transgenic chicken.